

AMENDMENTS TO THE CLAIMS

1. (Currently amended) A method for determining susceptibility of bacterial cells to an antibiotic comprising:

a¹ providing a test substance containing bacterial cells, the susceptibility of which to an antibiotic is sought;

contacting the test substance with a growth medium containing [an] the antibiotic, which is known to inhibit an operative enzyme of a bacterial biochemical pathway, to form a test substrate;

incubating the test substrate;

adding to the test substrate a histochemical reagent capable of generating a chromogenic compound as the result of interaction with the operative enzyme of the biochemical pathway[, if the operative enzyme is not inhibited by the antibiotic]; and,

observing the bacterial cells in the test substrate for the presence of the chromogenic compound, wherein:

if the chromogenic compound is observed, then the operative enzyme is not inhibited and the bacteria are not susceptible to the antibiotic whereas, if the chromogenic compound is not observed, then the bacteria are susceptible to the antibiotic.

2. (Original) The method of claim 1, further comprising:

contacting an aliquot of the test substance containing the bacterial cells with growth medium not containing the antibiotic to form a control substrate;

incubating the control substrate;

adding the histochemical reagent to the control substrate; and,

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observing the bacterial cells in the control substrate for the presence of the chromogenic compound.

3. (Original) The method of claim 1, wherein the bacterial cells are selected from the group consisting of *Pseudomonas*, *Eschericia*, *Streptococcus*, *Staphlococcus*, *Enterococcus*, *Enterobacteriaceae*, *Mycobacteria*, *Klebsiella* and *Haemophilis*.

4. (Original) The method of claim 1, wherein the operative enzyme is selected from the group consisting of transpeptidase, carboxypeptidase, tetrahydropteroic acid synthetase and dihydrofolate reductase.

5. (Original) The method of claim 1, wherein the antibiotic is selected from the group consisting of a β -lactam, a tetracycline, an aminoglycoside, a sulfonamide, a macrolide, a fluoroquinolone and trimethoprim antibiotic.

6. (Original) The method of claim 1, wherein the antibiotic is selected from the group consisting of ampicillin, cefazolin, cephalothin, ceftazidime, gentamycin, mezlocillin, oxacillin, penicillin, piperacillin, ticarcillin and trimethoprim.

7. (Original) The method of claim 1, wherein the test substrate, and control substrate if used, are incubated from about 1 to about 120 minutes.

8. (Original) The method of claim 7, wherein the test substrate, and the control substrate if used, are incubated from about 30 to about 90 minutes.

9. (Original) The method of claim 7, wherein the test substrate, and the control substrate if used, are incubated from about 10 to about 40 minutes.

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10. (Original) The method of claim 1 wherein the test substance comprises a body fluid.

11. (Original) The method of claim 10, wherein the body fluid is selected from the group consisting of serum, plasma, spinal fluid, phlegm, saliva, nasal discharge, ocular discharge and pus.

12. (Original) The method of claim 1, wherein the test substance is selected from group consisting of tissue and feces.

13. (Original) The method of claim 1, wherein the chromogenic compound is observable by the naked eye.

14. (Original) The method of claim 1, wherein the chromogenic compound is observed using instrumental means.

15. (Original) The method of claim 14, wherein the instrumental means comprises a light microscope, a UV spectrophotometer or a laser scanner.

16. (Original) The method of claim 1, wherein:
the antibiotic is trimethoprim;

the enzyme-catalyzed biochemical pathway is a folic acid synthesis pathway;
the test substrate is washed with pH6 phosphate buffer prior to contact with the histochemical reagent; and,

the histochemical reagent comprises tetra nitro blue tetrazolium (TNBT), magnesium chloride, sodium azide, nicotinamide diphosphate (NADP) and dihydrofolic acid.

17. (Cancelled) A kit for determining susceptibility of bacterial cells to one or more antibiotic(s) comprising one or more histochemical reagent(s), each of which is capable of generating a chromogenic compound by interacting with a bacterial biochemical pathway if an operative enzyme of that pathway is not inhibited by an antibiotic.

18. (Cancelled) The kit of claim 17, further comprising one or more antibiotic(s) that is(are) known to inhibit the activity of an operative enzyme of a bacterial biochemical pathway that one or more of the histochemical reagent(s) is capable of interacting with to form a chromogenic compound if the operative enzyme is not inhibited by the antibiotic.

19. (Cancelled) The kit of claim 17, further comprising a growth medium.

20. (Cancelled) The kit of claim 17, further comprising a fixing agent.

a) 21. (Currently amended) A method for determining the susceptibility of bacterial cells to a plurality of antibiotics, comprising:

providing a test substance containing bacterial cells;

providing a test plate having a plurality of wells, each well comprising a growth medium and a different antibiotic, wherein each antibiotic is known to inhibit an operative enzyme of a bacterial biochemical pathway;

placing an aliquot of the test substance containing the cells into each well;

incubating the test plate;

adding to each well a histochemical reagent, which is capable of generating a chromogenic compound as the result of interacting with [a] the operative enzyme of the biochemical pathway[, if an operative enzyme of that pathway is not inhibited by the antibiotic in that well]; and,

observing the bacterial cells in each well for the presence of the chromogenic compound, wherein:

in any well in which the chromogenic compound is observed, the operative enzyme is not inhibited and the bacteria are not susceptible to the antibiotic in that well whereas, in any well in which the chromogenic compound is not observed, the bacteria are susceptible to the antibiotic in that well.

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Cont 22. (Currently amended) A method for determining the susceptibility of bacterial cells to an antibiotic, comprising:

providing a test substance containing bacterial cells;

providing a test plate having a plurality of wells, each well comprising a growth medium and a different concentration of an antibiotic, which is known to inhibit an operative enzyme of a bacterial biochemical pathway;

placing an aliquot of the test substance containing the cells into each well;

incubating the test plate;

adding to each well a histochemical reagent, which is capable of generating a chromogenic compound as the result of interaction with the operative enzyme of the biochemical pathway[, if the operative enzyme is not inhibited by the concentration of antibiotic in that well]; and,

observing the bacterial cells in each well for the presence of the chromogenic compound, wherein:

Q in any well in which the chromogenic compound is observed, the operative enzyme is not inhibited and the bacteria are not susceptible to the antibiotic at the concentration in that well whereas, in any well in which the chromogenic compound is not observed, the bacteria are susceptible to the antibiotic at the concentration in that well.
